REMARKS/ARGUMENTS

By this Amendment, claims 1, 4, 6-8 and 15-16 are amended. Claims 1-20 are pending.

Favorable reconsideration is respectfully requested in view of the foregoing amendments and the following remarks.

Claim Rejection under 35 U.S.C. § 112

Claims 1-20 stand rejected under 35 U.S.C. §112, second paragraph as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. In particular, the Office asserts that the phrase "of from about" in claims 1, 4, 6, 8, 15 and 16, and the term "substantially" in claims 9 and 17 renders the claims vague and indefinite. This rejection is respectfully traversed.

The foregoing amendments deleting the term "about" from the claims obviate this rejection to the extent it was based on the alleged indefiniteness of "about".

With regard to the term "substantially", Applicants acknowledge that the specification does not provide an explicit definition. However, a person of ordinary skill in the art (a "POSA") would have understood from the original disclosure that the term "substantially" simply recognizes the practical limits of the diafiltration step recited in the claims. The POSA would have accordingly interpreted the expression "substantially all unreacted compounds and unconjugated polysaccharides are removed" as covering the removal of all such moieties, or at least as much as can be removed within the practical limitations of diafiltration.

Moreover, an April 7, 2010 search of the U.S. Patent and Trademark Office's U.S. Patent Collection database revealed that the term "substantially" occurs in the claims of 962,609 patents since 1976 (see attachment). This constitutes about 25% of the approximately 3.7 million patents (PN 7,6xx,xxx – PN 3,9xx,xxx) issued during this time. See, e.g., claim 20 of US 7,563,308, claim 7 of US 7,501,549 and claim 1 of US 5,756,716. Permitting the term to remain in the claims at issue would therefore be consistent with common PTO practices.

Accordingly, reconsideration and withdrawal of the indefiniteness rejection are respectfully requested.

Claim Rejections under 35 U.S.C. §103

Claims 1-4 and 8-14 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over US 6,800,728 (Schwartz), US 5,965,714 (Ryall et al.), and US 4,963,232 (Kuriyama et al.), as

evidenced by Behr et al. (Tetrahedron 59, pages 543-553). Claims 1-20 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over US 6,800,728 (Schwartz), US 5,965,714 (Ryall et al.), and US 4,963,232 (Kuriyama et al.), US 5,480,643 (Donovan et al.) and US 5,066,408 (Powell) as evidenced by Behr et al. (Tetrahedron 59, pages 543-553). These rejections are respectfully traversed.

Schwartz Fails to Disclose All Claimed Features

Schwartz relates to reagents and methods for crosslinking and immobilizing biomolecules, drugs and synthetic polymers. The reagents possess (i) a thiol or amino reactive group; and (ii) a hydrazino or oxyamino moiety. Conjugates and immobilized biomolecules are also provided. The motivation of Schwartz was to find a solution to conjugate different molecules and supports. Schwartz describes methodologies to expand the use of chemical reagents such as hydrazine, the derivatization of biomolecules, with the subsequent combination with other biomolecules, polymers, metals or drugs.

On the other hand, the claimed invention is directed to a method comprising the conjugation of biomolecules for **preparing a conjugate vaccine in commercial volumes** and includes all the steps necessary to obtain the conjugates from the reaction of activated protein with hydrazine dichloride and the polysaccharide activated by sodium periodate ($NaIO_4$) **in the presence of a reducing agent** (sodium cyanoborohydride - $NaBH_3CN$).

Thus, it must be emphasized that the present invention has developed all steps to produce, purify and control (Men A and C) conjugate vaccines. The downstream procedure was developed to optimize the steps of purification using tangential filtration in order to get good yields of soluble products without free polysaccharides. The established production procedures were reproducible. The physicochemical quality controls and immunogenic evaluation for Men C were consistent for the three final lots obtained.

No sodium cyanoborohydride

Although Schwartz describes the conjugation between oxidized polysaccharide and $NaIO_4$ and modified protein with hydrazine (see example 29), the reaction does not occur by the mechanism of reductive amination, i.e., the connection between the protein modified by hydrazine and polysaccharide activated by $NaIO_4$ occurs in the absence of reducing agent. **Thus, Schwartz fails to disclose or suggest the claimed feature of reacting the aldehyde-activated**

polysaccharide with the hydrazine-activated protein at a pH of from 5 to 7 in the presence of the reducing agent sodium cyanoborohydride.

Size Exclusion Chromatography is not Diafiltration

Additionally, in the purification step of the conjugate, Schwartz describes the use of size exclusion chromatography Superdex 200 column, contrary to the tangential filtration claimed by Applicants.

The tangential filtration process has advantages over size exclusion chromatography. Although both methods show similar performance when applied to processes of desalting or buffer exchange, the need for staggering (scaling) is achieved in an advantageous manner with the use of diafiltration in view of the increase in surface area filter, which is possible with the addition of membranes to the system and also the implementation of variations in processing time.

The sanitization of the diafiltration membranes is an easier process and, unlike the chromatography column, there is no need for re-packing after long use. In the case of chromatographic columns, there is a limiting factor. This limiting factor is the volume of sample to be processed, plus the cost, since it is directly proportional to the size of the column. In the case of molecular exclusion columns, for example, processing of large volumes of sample occurs with the fractionation of the sample and in multiple chromatographic cycles. In this case there is a need to combine the product of each cycle in order to obtain the final product. In the case of diafiltration, there is no problem with the processing of large volumes of material and, in addition, the process is performed in a continuous manner. A great advantage of such process is the possibility to concentrate the material in the same system used for the diafiltration, reducing its volume, which contrasts with reality that there is usually a dilution of about 40% in samples processed by size exclusion chromatography, resulting in the need of the concentration of the samples by ultrafiltration. Thus, the use of ultrafiltration for both desalting and concentration allows replacing the need for chromatography columns of size exclusion. See, e.g., Larry Schwartz, "Desalting and Buffer Exchange by Dialysis, Gel Filtration, or Diafiltration" http://www.pall.com/laboratory_42217.asp. (The Examiner will note from the concurrently submitted copy of this document, that it does not describe steps to obtain the conjugate to be used as a vaccine, that is, the steps of: (a) activation of the polysaccharide (reaction with $NaIO_4$,

generating aldehyde groups); (b) activation of tetanus toxoid with hydrazine and subsequent concentration and purification by tangential filtration in order to obtain at least 5 liters of the product; (c) conjugation of the activated polysaccharide with activated tetanus toxoid, purification and concentration by tangential filtration using membrane molecular cut-off of 100 kDa, in order to yield at least 2 liters of the product, wherein steps (a)-(c) are described in the patent application under examination.)

Protein Activation not at Acidic pH

This Schwartz patent describes the chemical modification of protein by using compounds such as hydrazine and oxiamine, at pH 7-9 (neutral to basic) for a period of 1 to 4 hours. The claimed invention, on the other hand, requires the activation of the protein by reaction with hydrazine dihydrochloride at an acidic pH (claim 1). In this reaction, hydrazide groups are introduced into the protein molecule by reaction with the carboxylic groups of amino acids: aspartic acid and glutamic acid by carbodiimide methodology.

In addition, Schwartz also did not report the possibility of obtaining conjugate vaccine in the lyophilized form (addition of sucrose and lyophilization) as described by Applicants.

The secondary art cited in each rejection fails to remedy all of the aforementioned deficiencies of Schwartz to teach the claimed invention.

Ryall et al. does not Teach Neutralizing Step

With respect to Ryall et al., there is a inconsistency in relation to the use of adipic acid dihydrazide (ADH), since this reagent is used to activate the polysaccharide, which was previously "oxidized" by the action of peroxide hydrogen, by reaction with carbodiimide - EDAC (see column 2, lines 20 to 38, column 3 lines 40 to 45). In Applicants' invention, the reagent ADH is used to neutralize the aldehydes present on the activated polysaccharide which did not react with activated tetanus toxoid during the conjugation reaction (see description and claim 15 of the present invention).

Ryall et al. does not Teach Activation with $NaIO_4$

Further, the patent of Ryall et al. describes the activation of the polysaccharide from the reaction with hydrogen peroxide (30 to 80°C) (see column 5, line 23), where the chains of molecules are cleaved in a random fashion, resulting in the formation of carboxylic acid and aldehyde groups in the reducing end (see column 7, lines 48 to 50). Applicants disclose and

claim the activation of the polysaccharide with $NaIO_4$, which occurs selectively on the cleavage of the link between vicinal carbon attached to hydroxyl groups. In addition, the activation is also specific, resulting in the formation of terminal aldehyde groups.

Purification Step Distinctions

Ryall et al. describe the purification of the conjugate obtained from the reaction between the activated polysaccharide with hydrogen peroxide and derivatized by the introduction of amine groups (by reaction with ADH, using carbodiimide method), with the carrier protein. In the case of the diphtheria toxoid, the purification occurs in two basic steps: first purification of the conjugate is achieved by saline precipitation using ammonium sulfate concentration of 70% w/v. Then, Ryall et al. describe the use of diafiltration using a membrane (Omega modified polyethersulfone, screen channel unit cassette) with a molecular weight cutoff of 30 kDa (see example 3).

The Ryall et al. purification procedure differs from that of Applicants' invention, which is carried out by tangential filtration using a filter with a higher molecular weight cutoff (100 kDa is presently preferred) to obtain the at least two liters of product called for in claim 1. It must be pointed out that the structural characteristics of the conjugate obtained from the methodology described in the application was the guide for the selection of molecular weight cutoff of the membrane used in the purification step by diafiltration.

Conjugation Step Distinctions

The methodology described by Ryall et al. for conjugation between the polysaccharide and carrier protein, differs from that of Applicants' invention, as the polysaccharide, besides being oxidized/depolymerized, is derivatized with ADH by carbodiimide methodology. Unlike Ryall et al., Applicants oxidize the polysaccharide using $NaIO_4$ when aldehyde groups are generated.

With respect to the conjugation reaction itself, the patent of Ryall et al. describes that the link between the derivatized polysaccharide and carrier protein derivatized with cystamine, occurs in the presence of EDAC (conjugate by the carbodiimide method), at a temperature of 22 to 23°C for 22 to 24 hours, i.e., the chemical bonding occurs between the amine groups introduced into the polysaccharide and the carboxyl groups present in the protein (see column 8, line 54 to 58 and examples 3 and 9). This reaction occurs in the absence of $NaBH_3CN$.

On the other hand, Applicants employ the conjugation method of reductive amination (see, e.g., Jennings & Lugowski, 1981), where aldehyde groups generated in the polysaccharide are conjugated to amine groups introduced into the protein in the presence of a reducing agent ($NaBH_3CN$) at a temperature of 22 to 45°C overnight.

Kuriyama et al. does not Teach Use of Hydrazine Dihydrochloride

With respect to patent Kuriyama et al., the description of methodology for obtaining hydrazine with reduced content of organic carbon (impurities) does not correlate with the use of hydrazine dihydrochloride, which is a commercial reagent with a purity degree suitable for obtaining conjugate vaccines.

Additional References do not Remedy Aforementioned Deficiencies

Regardless of whether or not the Office is correct that Donovan et al. and Powell et al. teach the use of sodium carbonate as a buffer, these references still fail to remedy the aforementioned deficiencies of the other references to teach all the limitations of the claimed invention.

Accordingly, reconsideration and withdrawal of the obviousness rejections are respectfully requested.

For at least the reasons set forth above, it is respectfully submitted that the above-identified application is in condition for allowance. Favorable reconsideration and prompt allowance of the claims are respectfully requested.

Should the Examiner believe that anything further is desirable in order to place the application in even better condition for allowance, the Examiner is invited to contact Applicants' undersigned attorney at the telephone number listed below.

Respectfully submitted,

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